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STUDIES

PHYSIOLOGICAL SERIES

No. 33: OBSERVATIONS ON THE GLYCOGEN CONTENT OF CERTAIN INVERTEBRATES AND FISHES, BY L. G. KILBORN AND J. J. R. MACLEOD

(REPRINTED FROM THE QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY, VOL. XII)

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OBSERVATIONS ON THE GLYCOGEN CONTENT OF CERTAIN INVERTEBRATES AND FISHES.

By L. G. Kilborn and J. J. R. Macleod. (From the Marine Biological Station, Nanaimo, B.C., and the Department of Physiology, The University of Toronto.)

(*Received for publication 27th December* 1919.)

Throughout the animal kingdom, from the Amœba to Man, carbohydrate is known to be an important constituent of the organism. It is most characteristically represented within the cells by the polysaccharide glycogen, and in the circulating fluids by glucose. In the higher vertebrates the percentage amount of glycogen in the different organs and tissues varies considerably, being usually greatest in the liver. The amount is, however, closely related to the time of feeding and the nature of the food. In the circulating fluids of the animal, on the other hand, there is apparently a tolerably constant percentage of glucose, if the temporary increase which follows immediately upon the ingestion of food (the postprandial rise) be discounted.

Here and there in the animal kingdom, however, it has been asserted that very little, if any, glycogen (certain molluscs) or glucose (Selachians) is present. If this should prove to be the case, it would mean either that some other carbohydrate is substituted for glucose and its polysaccharide—such as a pentose,—or that metabolism proceeds in these animals in the absence of any of the higher carbohydrates. Scrutiny of the published researches upon which these generalisations depend shows that the methods employed have been very unequal in value both qualitatively and quantitatively. For the detection of glycogen the microchemical reaction with iodine has been extensively used, and for the quantitative determination of this substance usually the somewhat uncertain method of Brücke-Külz; it is only here and there that the more certain method of Pflüger has been employed. For the detection and measurement of the glucose it has been usual to utilise the reducing power, after precipitation of the proteins by various methods.

In consideration of these facts, it was thought advisable to seize the opportunity afforded by several weeks' residence at the Marine Biological Station (situated on the east coast of Vancouver Island, at Nanaimo, B.C.) to determine by standard methods the relative amounts of the above-mentioned carbohydrates in selected varieties of marine animals. In planning such an investigation it was recognised that it is decidedly risky

to assume that methods for the isolation and determination of glycogen and soluble reducing carbohydrate, which have been shown to be reliable for higher land mammals, must also necessarily be so in the varied conditions met with in the lower marine animals. The chemical structure of the circulating fluids and of the tissues might, for example, be so far different as to interfere with the proper removal of the proteins prior to estimation of the glycogen or glucose. Notwithstanding this possible source of fallacy, it was thought that the first step in the investigation should consist of determination of the above substances by the accepted standard methods, reserving for future research a more detailed investigation of the causes for deviation in the results.

A general review of the work bearing on the distribution of glycogen in the animal kingdom is given by Pflüger (1) (up to 1903) and by Biedermann (2) (up to 1911).

Much of the work referred to in these reviews is microchemical in nature, and may for the present be disregarded. Of the work in which glycogen was isolated by chemical methods, the following references have more or less bearing.

Amongst the Protozoa, glycogen has been isolated by the Brücke Külz method in Infusoria (Barfurth (3)), particularly in a culture of Glaucoma scintillans. The isolated glycogen gave the characteristic reaction with iodine, and yielded a reducing substance after hydrolysis with mineral acid. The starch-like granules, called paraglycogen, which have been described in the protoplasm of certain of the Infusoria (Gregarina), are apparently not ordinary glycogen, for although they give a brown reaction with iodine, they are insoluble in cold water, and are not hydrolysed to reducing sugar by saliva. By prolonged hydrolysis with weak acid, however, reducing substance is produced (Bütschli (4); see also Maupas (5)).

The so-called "Glanzkörper," found particularly in Pelomyxa, are also often considered as being closely related to glycogen. They are readily formed in the cell when the animal is fed with polysaccharides (starch, celluiose), they give a reaction with iodine like that given by glycogen, and reduction occurs after hydrolysis of the bodies of animals containing them. Apart from these observations, there appears to be no chemical evidence that the bodies are really glycogen (Greeff (6)).

Biedermann, in summing up the work on the Protozoa, states that the digestion of starch proceeds in these animals much in the same way as in the Metozoa, and that storage of the digested starch in the form of glycogen certainly occurs in Pelomyxa and often in the Ciliata and Gregarina (loc. cit., p. 386).

In Euglena, which represents the highest of the Flagellata, a substance called paramylon has been extensively studied by microchemical methods, and has been found to vary greatly in amount according to the pabulum upon which the Euglena is grown, and whether or not it is associated with

chlorophyll. When chlorophyll is present in the cell paramylon is abundantly formed and can be dissolved out by weak alkali and precipitated by acid alcohol. It gives no colour with iodine, is insoluble in water, and when hydrolysed by acid yields a reducing substance which ferments with yeast. Diastase is said not to digest this material, however (Bütschli (7)).

It is stated by Pflüger (op. cit.) that glycogen has been described as present in certain of the Echinodermata (asteroids, holothurians) and in sponges. It has been detected by microchemical methods in the eggs of insects and molluscs. As much as 2 per cent. of glycogen, apparently identical with that prepared from rabbit liver and from oysters, was isolated by Harden and Young (8) from washed pressed yeast.

Taking the observations as a whole, it is certain that polysaccharide material, which is more or less closely related to glycogen, is a common constituent of the cells of the lowest animals as well as of such plants as yeast, which do not synthetise sugars through the action of chlorophyll.

From certain Ascaridæ and also from Tænia, Kobert (9) was able to prepare a strongly opalescent watery solution reacting with iodine. On standing the glycogen became digested, although the extract did not cause digestion of added starch. This would seem to point to the existence of a specific glycogenase. Weinland (10) found that about one-third of the weight of dried Ascaris consisted of glycogen; in Tænia even a higher amount, namely, one-half of the dry weight, consisted of this material. In Distoma hepaticum also a large amount of glycogen was present. It is interesting to note in passing that this glycogen was found quickly to disappear when the worms were kept under anaerobic conditions. Glycogen is also present in considerable amount in the earthworm (Lesser (11)). In the so-called chlorogogen cells which surround the alimentary canal very large amounts of glycogen have been described: the leucocytes in the body fluids also contain it, but it is said to be absent from the muscles (Cuenot (12)).

A considerable amount of attention has been paid to the carbohydrate present in the digestive gland (liver) of various Mollusca. Biedermann gives an adequate review of the work. Of particular importance in the present connexion is the work of Barfurth (cf. p. 342, op. cit.), who found in air-breathing gastropods after feeding for one to three days with bread from 3·4 to 6·4 per cent. in the liver and 3·3 per cent. in the foot. He concludes that the liver of gastropods plays almost as important a part in storing glycogen as does that of mammals.

In those snails (Helix) in which there is relatively a large amount of connective tissue, the glycogen was found to be stored mainly in the plasma cells that are present in this tissue, whereas in snails (Limax) in which there is little connective tissue the plasma cells soon become filled and the glycogen is present mainly in the columnar epithelial cells both of the intestine and of the biliary passages (Barfurth, op. cit., p. 328).

These observations are of interest in connexion with the observation first made by Claude Bernard, and subsequently confirmed by Barfurth and Biedermann, to the effect that the contents of the stomach and upper portion of the intestine, after they have been partially digested, move in and out of the liver passages. Absorption occurs through the cells both of the intestine and biliary passages, and when it is completed the fluid left in the stomach is colourless, though at first it was coloured by the secretion of the liver. It is important to note these facts in connexion with the present investigation, because they indicate that we must be careful not to conclude that any glycogen found in the liver of molluscs is necessarily present in this organ as such. As a matter of fact, several investigators (Frentzel, Röhmann (13), Bottazzi (14)) have been unable to detect any glycogen either by microchemical or biochemical methods in the livers of Aplysia and Arion. The two observers last mentioned have, independently, found in Aplysia (the sea-hare), in place of glycogen, a pentose-yielding substance (pentosan), the precise identity of which is uncertain. Röhmann thought this pentosan to be derived from undigested residue of the food of the animal, which consists of an Alga (Ulva lactuca) and which contains pentosan as well as starch. The residue finds its way into the biliary passages as described above. Bottazzi agrees that it is derived from the pentosan of Ulva, but believes that it has become partly broken up so as to form an acid substance (acide pentosique) which takes the place of mineral acid, which is absent in these molluscs.

M. Henze (15) could not find a trace of glycogen or of water-soluble carbohydrate in the muscles or liver of Octopods. Some evidence was obtained, however, that a glucoprotein may represent the carbohydrate storage material in these animals.

Further attention has been paid by Henze and Starkenstein (16) to the supposed absence of glycogen in the liver of molluscs. They point out that if this conclusion is confirmed it would indicate that, in so far as their carbohydrate metabolism is concerned, certain marine molluscs occupy a peculiar position in the animal scale, glycogen being apparently present in all others. They show that a certain error may be incurred in estimating glycogen in the organs of sea animals on account of the presence of the salts of sea water, particularly Mg. The $Mg(OH)_2$ which is formed with KOH adsorbs some of the glycogen, and when alcohol is subsequently added a precipitate is formed from which boiling water does not dissolve out all the glycogen. These authors also point out that an error of another nature may be incurred because of the presence of glucosamin derivatives and of pentosans, unless the tissue be treated with the strong alkali for a sufficient length of time to ensure the entire destruction of these substances. This matter will be referred to again later. It is recommended by Starkenstein and Henze that the above-mentioned sources of inaccuracy be circumvented by prolonged heating with KOH,

then adding only one volume instead of two of alcohol to precipitate the glycogen, and finally hydrolysing the alcohol precipitate directly without first of all dissolving it in water. They offer no experimental proof, however, that their method is any more accurate than the original method of Pflüger. They found as much as 1·25 grm. glycogen in the liver of Aplysia lenescona.

Among the Crustacea it was found by Hoppe-Seyler (17) that there was still some glycogen in the liver of 5–6-year-old crayfish (Flusskrebsen) after starvation. Claude Bernard had previously found the amount to vary more or less in relationship to the time of moulting, being greatest just prior to this period. At the time of moulting Kirsch (18) found the entire body (of the fresh-water crayfish) to contain 0·82 per cent., whereas four months before this period it contained only 0·08 per cent.

Until recently very little information existed concerning the presence of glycogen in the fishes. That some at least is present in the tissues of marine fish had been shown by Cl. Bernard, Pavy, Brücke, and others. It was stated by Bernard that this glycogen is unusually resistant to the influence of post-mortem changes, and that it does not readily disappear during hunger. During asphyxia, however, the glycogen rapidly disappears.

Schöndorff and Wachholder (19) adequately review all these older investigations, and contribute numerous observations of their own in which the amount of glycogen, determined by Pflüger's method, in a large variety of fishes caught in fresh water is given. The percentage amount varied between 2·5 and 12·94. Prolonged hunger was found to cause considerable reduction in the amount of glycogen in such fish as the pike (Esox lucius), which remain active, but to cause only slight reduction in the carp (Cyprinus carpis) and other fish, which hide themselves away in the mud during winter. Post-mortem glycogenolysis did not appear to proceed as rapidly as in Mammalia. The estimations were made by measuring both the reducing and the rotating power of the hydrolysed glycogen precipitates, the close correspondence of the results obtained by the two methods indicating that the material is chemically identical with that present in mammalian tissues.

Methods.

Glycogen.

The organ or tissue was cut into small pieces, pressed between filter paper, weighed, and dropped into 95 per cent. alcohol, in which it was shipped from the station to Toronto, the journey occupying about a week.

It is possible in the case of one or two specimens of muscle (e.g. siphon muscle of clam) that there was relatively too small a quantity of alcohol entirely to prevent a certain amount of glycogenolysis. The muscle in

these cases was decidedly compact, and may not have been sufficiently cut up to ensure immediate penetration by the alcohol. This possible source of error was, however, not incurred in the vast majority of cases. The alcohol-preserved material was pressed between filter paper and again weighed. It was then heated for three hours with 60 per cent. KOH and the glycogen determined by Pflüger's method.

As already mentioned, Starkenstein and Henze state that glycogen determination by the Pflüger method is inaccurate in the presence of sea water because of adsorption of the glycogen by the $Mg(OH)_2$ and $Fe(OH)_3$ that are formed when KOH is added. This adsorbed glycogen after precipitation with alcohol does not become completely dissolved in boiling water.

By dissolving equal amounts of pure glycogen in sea water and in distilled water and then carrying out the usual Pflüger process with both, we have confirmed the observations of the above authors. We do not believe, however, that the organs and tissues of sea animals contain a sufficiency of salts precipitable by KOH to make any significant error in the glycogen determinations. This conclusion is based on the following observations:—

The liver of a rabbit was cut in small pieces and quantities of 20 grm. each were added to equal volumes of (a) sea water, (b) 0·9 per cent. sodium chloride solution, in which they were thoroughly ground in a mortar. To each of the resulting suspensions equal volumes of 60 per cent. KOH solution were added and the glycogen content determined by the Pflüger process. The following results were obtained: in (a) 0·675, (b) 0·784 per cent. Even in the presence of a very large excess of sea water—very much more than could be present even in tissue which has not been pressed between filter paper—the error incurred is not excessive.

In order to see whether any large yield of glycogen would be obtained when the alcohol precipitate was directly hydrolysed, 28 grm. of oyster (from which the excess of sea water had been removed by pressing between filter papers) was treated with KOH and the glycogen precipitated with alcohol and divided into two portions, *a* and *b*. The precipitate in *a* was dissolved in boiling water in the usual manner (i.e. on the filter paper, and the filtrate hydrolysed), whereas that in *b* was removed from the filter to a flask by a fine stream of water and directly hydrolysed. The following results were obtained: in *a*, 0·569 grm. glucose, in *b*, ·694 grm. glucose. There is therefore evidence that it is necessary to modify the Pflüger process to the extent that the glycogen precipitates are washed from the filter paper into a flask and directly hydrolysed. This procedure has not been followed in the present investigation, partly because Starkenstein and Henze's work did not come to our notice until the work was nearly completed, and partly because the precipitates in many cases were of such a nature that their removal from the filter

paper would have been practically impossible. It is considered that the error incurred on this account is insignificant, especially since very little of the salts of sea water could have been present in the tissue used for the estimations.

Results.

Glycogen.

These are given in percentages both of the original and the alcohol-dried material. Although neither result in itself is more than approximate because of the impossibility of removing all the adherent moisture or alcohol by means of filter paper, they are sufficiently close to be used for the purpose in view, namely, to determine the relative amounts of glycogen in the tissues of different animals.

The observations were made on specimens from the following groups of animals:—

The Echinodermata (Asteroidea).
The Mollusca (Lamellibranchiata).
The Arthropoda (Crustacea).
The Fishes (Elasmobranchii and Teleostomi).

I. THE ASTEROIDEA.

Table I.—Percentage of Glycogen (as Dextrose) in the Hepatic Cæca of Star-fishes.

No.	Species.	Weight of material (grm.).	Glycogen percentages		Remarks.
			Calculated for original material.	Calculated for alcohol-preserved material.	
M	Pisaster ochrac[illegible] . .	41·9	1·23	1·52	1 large specimen.
T	„ „ . .	14·2	...	0·268	2 small specimens.
E_1	„ „ . .	16·6	...	0·478	3 „ „
H_1	Pisaster brevispinus .	6	...	0·232	1 specimen; liver very small.
L	Pycnopodia heliantoides .	34·3	0·62	0·93	From 1 specimen.
I_1	„ „ .	24·6	...	0·60	
S	Evastarius Troschelli .	11·95	...	0·90	
F_1	Luidia foliata . . .	22	...	0·461	Mud star-fish.
G_1	„ „ . . .	18	...	0·403	

The variable amount of glycogen found in these animals is probably dependent upon whether or not they have recently been feeding, the percentage of glycogen being plainly proportional to the size of the cæca. The glycogen precipitate in M was further examined. After redissolving in boiling water it was found to give a characteristic reaction with iodine, and on hydrolysis to yield a sugar which fermented readily with yeast.

II. THE MOLLUSCA.

TABLE II.—PERCENTAGE OF GLYCOGEN IN VARIOUS PARTS OF THE BODY OF THE HORSE CLAM (SCHIZOTHOERUS NUTTALLI).

No.	Organ or tissue.	Weight of material (grm.).	Glycogen percentages	
			Calculated for original material.	Calculated for alcohol-preserved material.
A	Digestive gland .	31·2	...	(a) 0·31
N	„ „ .	22·5	...	(b) 1·56 *
F	Muscle of siphon .	56·1	...	(a) 0·077
Q	„ „ .	30	...	(b) 0·952
C	Muscle of foot .	54·25	...	(a) 0·46
P	„ „ .	19·4	...	(b) 1·70
B	Adductor muscles .	63·5	...	(a) 0·40
O	„ „ .	20·6	...	(b) 2·67 ?
R	Posterior adductor .	17·1	...	(b) 2·75 ?

* Hydrolysed glycogen fermented with yeast.

The estimations marked (a) were made on clams that had been kept for some time (1-2 weeks) in a sack immersed in the sea at the wharf. Those marked (b) were made on clams that had been kept only a day or so after digging them up.

These observations were carried out on two batches of clams. After being dug from a sandy beach at low tide the clams were placed in a sack which was then immersed in the sea at the end of the wharf, specimens being removed from time to time for analysis. The clams of the first batch *a*, although receiving no food, remained alive in the sack for over two weeks, but those of batch *b* did not survive in the sack for more than a few days, and they disintegrated very rapidly after death. The estimations, the results of which are recorded above, of group *a* were made after one week in the sack; those of group *b*, on the other hand, were made within a day or two after the clams were collected. It is possible that variations in the state of the material accounts for the persistently higher percentages of glycogen in group *b* than in group *a*. In order to make a comparison of the glycogen in the various organs and tissues, it is necessary, therefore, to take the results of each batch, *a* or *b*, separately.

In batch *a* the largest amount of glycogen was found in the muscle of the foot, with very nearly the same amount in the adductor muscles. Decidedly less was present in the liver, and only a trace in the muscle of the siphon. More interesting results are those of batch *b*. In this case the adductor muscles contained a higher percentage of glycogen than we can find recorded for muscle. (The highest given by Pflüger in "Das Glykogen" is 2·44 per cent. This was found by Aldehoff in the glutæus maximus of the horse.) We are, however, not certain that it is typical glycogen that is responsible for all the high percentage found in the case of the adductor muscles. There are several reasons for this doubt: first,

the precipitate produced by alcohol did not settle as ordinarily, but required the addition of a considerable amount of sodium chloride to cause it to do so; second, on neutralising the hydrolysed solution of the precipitate and fermenting with yeast only a small quantity of gas collected, whereas the precipitates from the liver, when similarly treated, give a much larger amount of gas. We had not sufficient material with which to investigate this question further. These muscles are entirely different in structure from the muscle of the foot or siphon, being composed of large bundles of very pale substance.

There is also a high percentage of glycogen in the muscle of the foot, although this muscle can be used but seldom after the clam has assumed its more or less permanent position in the sand. The glycogen in the siphon muscle is decidedly less (0·95 per cent.); this muscle is used to hold the tubes open as well as to retract the siphon on the approach of danger.

It will be observed that a considerable amount of glycogen was found in the liver. This is of interest because of the belief already referred to (p. 320), that there is no glycogen in the liver of certain molluscs (Aplysia and Arion). That the material found by us was glycogen, as ordinarily understood, was shown by its general behaviour towards strong alkali, iodine, etc., and by the fact that the hydrolysed glycogen readily fermented with yeast and gave typical glycosazone crystals.

It will be recalled that several observers (Frentzel, Röhmann, Bottazzi) have averred that there is no glycogen in the liver of certain other species of the Mollusca. These workers found evidence of the presence of pentoses and methylpentoses, and they have suggested that polymerised forms of these may replace glycogen. Since they did not consider the likelihood that the pentoses were derived from inosinic acid or guanylic acid, the conclusions cannot be given much weight. As a matter of fact, it has been shown by Mr C. E. Berkeley, working in the biological station, that the pentose present in the tissues of closely related species is derived from one or other of the above-mentioned nucleotids.

The percentage of glycogen is notably different in the small crab (Cancer productus) and in the lobster. The difference is probably dependent upon feeding conditions. The lobsters were shipped from St Andrews, on the New Brunswick coast, to Toronto in moist seaweed, the first batch in September, when the weather was warm, and the second batch in November, when it was very cold. The difference in temperature does not appear to influence the results. The larger crabs were caught at St Andrews in November and transported to Toronto. They were almost dead when received. Although they contain much less glycogen in proportion than the smaller crabs, it will be noted that there is decidedly more in the liver and in muscle than in those tissues in the lobster.

A comparison of the glycogen content of different muscles in the lobster is of interest. In the muscles of the back and tail the average for four

III. THE ARTHROPODA.

TABLE III. PERCENTAGE OF GLYCOGEN IN VARIOUS PARTS OF THE BODY OF CRUSTACEA (CANCER PRODUCTUS AND HOMARUS AMERICANUS).

No.	Species.	Organ or tissue.	Weight of material (grm.).	Glycogen percentages	
				Calculated from original material.	Calculated from alcohol-preserved material.
D	Cancer productus	Liver	...	...	1·39
D_1		„	7·37 *	...	0·37
E		Muscles	24·65	...	0·87
p. 34	Homarus . .	Liver	35·64	0·78	
„ 36		I. Heart	2·37	0·91	
		II. „	1·94	1·42	
„ 40		I. Muscle (tail)	55	0·36	
„ 41		„ (claw)	50	0·17	
„ 44		II. Muscle (claw)	45·7	0·10	
„ 48		II. „ (tail)	42·6	0·32	
1 (p. 3)	Homarus I.	Heart (of six lobsters) †	7·32	0·85	
(p. 4)		Liver	30	0·05	
		Muscle (back)	20	0·31	
		„ (claw)	20	Trace	
	Homarus II. .	Liver	20	0·13	
		Muscle (red claw)	20	0·17	
		„ (white claw)	20	0·17	
		„ (back)	20	0·30	
	Homarus III. .	Muscle (back)	20	0·30	
	Cancer irrotatus	Heart ‡	2·77	0·51	
		Liver	24·47	1·00	

* Weight after preservation in alcohol.

† The hearts of six lobsters were used for this determination. The weight of the animals and of the heart in each specimen was as follows: (1) 2000 grm. and 1·95 grm., (2) 1650 grm. and 1·35 grm., (3) 1150 grm. and 1·00 grm., (4) 1150 grm. and 1·02 grm. (5) 1100 grm. and 1·00 grm., (6) heart 1·00 grm.

‡ The hearts of five specimens were used.

lobsters was 0·32 per cent., being remarkably close to this figure for all of the animals. The pale muscle of the claw gave an average for three lobsters of about 0·15, only a trace being found in that of a fourth animal. There is therefore decidedly more glycogen in the tail muscle. Of greater interest are the results obtained for the heart. Being very small (weighing only 1·95 grm. in a lobster weighing 200 grm.), it was necessary to collect the hearts of several animals to make the analysis with any precision. The average of three determinations on different collections of material was 1·06 per cent., the lowest value being 0·85 per cent. The relatively high

percentage of glycogen in this primitive form of heart is interesting in view of the fact that a similar result was obtained in the heart of fishes. Further reference to the significance of this observation will be found in connexion with the latter.

With regard to the liver, the percentage amounts of glycogen varied considerably (viz. between 0·05 and 0·78 per cent.), thus contrasting with the muscles, where the amounts were tolerably constant. No doubt, as in mammals, the glycogen content of this viscus depends primarily on the activity of digestion, which it is presumed must have varied for different individuals. The largest result was obtained in a specimen caught in September.

IV. THE FISHES.

TABLE IV.—PERCENTAGE OF GLYCOGEN IN VARIOUS PARTS OF THE BODY OF FISHES CAUGHT DURING JULY AND AUGUST.

No.	Species.	Organ or tissue.	Weight of material (grm.).	Glycogen percentages	
				Calculated from original material.	Calculated from alcohol-preserved material.
H	Elasmobranchii (Squalus Sucklii), dog-fish	Liver	44·2	0·057	0·069
I	"	"	20	0·16	0·209
V	"	" *	19·8	None	None
K		Muscle (body wall)	50	0·018	0·025
W		Muscle *	19·8	None	None
G		Heart	7·3	0·447	0·847
U		" *	4·6	...	0·172
Z	Chimæra (rat-fish) . .	Liver †	16·7	None	
A_1			15·2	"	
	Teleostomi (Cyprinus carpio), carp	Muscle	10·3	"	
p. 95		(a) Liver	3·44	(a) Trace	
96		(a) Muscle	50	(a) "	
99		(b) "	50	(b) 0·021	(b) 0·028
23		Liver	10	6·50	
24		Muscle	20	0·29	
25		Liver	10	5·60	
26		Muscle ‡	20	Trace	
98	Christivomer Namaycush (lake trout)	(a) Liver	26·6	None	
98		(b) "	45	0·055	
97		(a) Muscle	13·91	Trace	
97		(b) "	11·70	"	

* Dog-fish caught in nets and kept in small tank for some days. Otherwise the dog-fish were freshly caught by line from the end of the wharf.

† Dead some time.

‡ Fish dead at least 24 hours.

The outstanding feature of most of these results is the absence or the small amount of glycogen. The organs were removed for chemical investigation immediately after the fish were caught: except in the case of certain of the dog-fish and the rat-fish, in which the fish, entrapped in nets, were brought to the station in a half-dead condition in the tank of the boat. The results stand out in sharp contrast with those of Schöndorff and Wachholder already referred to, in which the liver and muscles of a long list of fresh-water (Rhine) fishes show in general as high percentages of glycogen as have been found in any animal. The only fundamental difference between the two series of observations is with regard to the time of year at which the fish were caught. The difference cannot at all depend on whether the fish was caught in fresh or in sea water, since in our series fish from both were examined. The carp numbered 21–26 inclusive were obtained in November from a local dealer. Two were alive when brought to the laboratory. It will be noted that decidedly more glycogen was found than in the fishes examined in August (Nos. 95–99). Although not conclusive, this supports the view that the glycogen content of fishes is very low in the summer months and high in winter.

The very low glycogen content of fish caught in summer, as compared with the decidedly high percentage in winter, is in conformity with similar observations in Amphibia and hibernating mammals. Thus Athanasiu (op. cit.) found in the liver of Tusca 2·77 per cent. and 4·35 per cent. in July, and 8·21 per cent. and 7·52 per cent. in October and November. Lesser (20) and Bleibtreu (21) have also demonstrated a great decrease of glycogen in the liver and other tissues of frogs during the spring and summer months (accompanied by a steady increase in the ovaries) and its great abundance in the late autumn and early winter.

One other fact deserves attention in connexion with these results, namely, the relatively high percentage of glycogen in the heart of the dog-fish. In hearts from two recently caught dog fish 0·447 per cent. (of fresh weight) of glycogen was found, when the livers of the same fishes contained only 0·057 per cent. and 0·16 per cent., and the muscles of the body wall only 0·018 per cent. In hearts from other dog-fishes that had been caught for some time and were partially asphyxiated, 0·172 per cent. (of alcohol-dried tissue) glycogen was found when no trace could be detected in the liver or muscles.

The presence of a large amount of glycogen in heart muscle has been known for some time (cf. Pflüger, loc. cit.), and has recently been confirmed by Cruickshank (23), who found in the heart of the dog from 0·300–0·631 per cent. It has further been shown by histo-chemical methods that the conducting tissues (the A–V node and A–V bundle) are especially rich in glycogen, a fact which is of particular interest in the light of our observation that there is a very high percentage in the primitive heart of the dog-fish, and also in that of the lobster. We were unfortunate

in failing to secure more material upon which to follow up this interesting observation.

Conclusions.

By Pflüger's method the following percentage amounts of glycogen were found present in the digestive gland (hepato-pancreas) of representative species from various aquatic phyla:—Asteroidea, 0·232 to 1·52; Lamellibranchiata, 0·31 to 1·56; Crustacea, 0·05 to 1·39; Elasmobranchii, none to 0·21; Teleostomi, none to 6·5.

The varying amounts are apparently dependent partly on feeding conditions and partly on the season.

In the muscles the following percentage amounts were found:—Lamellibranchiata, 0·077 to 2·67 (the latter value was obtained in the adductor muscles); Crustacea, trace to 0·36; Elasmobranchii, none to 0·018; Teleostomi, none to 0·29.

In all cases where it was possible to secure a sufficient amount of heart muscle the glycogen content was found to be several times greater than that of the other muscles, and sometimes greater than that of the liver; thus in the lobster from 0·85 to 1·42 per cent. was found in the heart, compared with a maximum of 0·36 per cent. in the muscles.

In several cases the glycogen was found to yield a yeast-fermentable sugar after hydrolysis, but in others there is some evidence that a certain proportion of the "reducing material" was due to other substances.

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